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in

Molina Alcaide E. (ed.), Ben Salem H. (ed.), Biala K. (ed.), Morand-Fehr P. (ed.). Sustainable grazing, nutritional utilization and quality of sheep and goat products

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 67

2005
pages 423-428

Traceability of grass-feeding in small ruminants meat and milk: A review

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SUMMARY – Supplying the consumers with guarantees concerning the food given to animals necessitate reliable methods to characterize meat and milk. This paper reviews the current state of knowledge concerning traceability of grass-feeding in small ruminant meat and milk. It presents the potential tracers and the different methods that are being investigated, and the recent results that have been obtained. Plant compounds such as carotenoids and terpenes, and animal metabolites such as 2,3-octanedione, skatole and fatty acid composition are potential tracers of grass-feeding in meat and milk. Global approaches, especially near infra-red spectroscopy are under study. These techniques already allowed to discriminate products obtained in contrasted feeding conditions (grazing at pasture vs concentrate-based diets indoors). Intermediate situations may be more difficult to recognize and should require the combined use of tracing methods. In particular, the persistence of tracers when animals are stall-fed a concentrate-based diet after pasture and its implications for traceability purposes are discussed.

Key words: Tracer techniques, grass, grazing sheep, goat.

RESUME – *"Traçabilité de l'alimentation à base d'herbe dans la viande et le lait des petits ruminants". Apporter aux consommateurs des garanties concernant l'alimentation des animaux d'élevage nécessite de disposer d'outils fiables de contrôle. Ce texte fait le point sur l'état des connaissances concernant la traçabilité de l'alimentation à l'herbe dans la viande et les produits laitiers des petits ruminants. Les méthodes en cours de développement font appel soit à des traceurs d'origine végétale tels que les caroténoïdes et les terpènes, soit à des métabolites de l'animal dont la présence ou les proportions sont caractéristiques de l'alimentation : 2,3-octanedione, scatole et composition en acides gras. Des méthodes globales telles que la spectroscopie dans le proche infra-rouge sont également en cours d'élaboration. Ces techniques ont déjà permis la discrimination de produits obtenus avec des types d'alimentation contrastés (herbe au pâturage vs aliment concentré et foin en bergerie). Les situations intermédiaires sont plus difficiles à caractériser et nécessitent l'utilisation conjointe de plusieurs traceurs. La persistance des traceurs lorsque les animaux sont nourris en bergerie après une période de pâturage et ses conséquences en termes de traçabilité sont notamment discutées.*

Mots-clés : *Traçabilité, herbe, pâturage, ovins, caprin.*

Introduction

There is an increasing consumer demand for clear information regarding the food supplied to animals, and an increasing consumer interest in the green image of animal products, grassland-based food products being considered as safe, natural and respectful of animal welfare. Control structures also ask for controlling tools, in order to objectively guarantee that the specification commitments have been correctly fulfilled. Being able to trace the food given to animals is therefore a major challenge for scientists, control and commercial structures and farmers. Recent efforts have been made to develop analytical tools to quantify specific compounds in the product or the animal tissues that can act as tracers of the type of food given to animals. This paper mainly refers to sheep and goats, but see Martin *et al.* (2002) for dairy cows, and Cornu *et al*. (2001a) and Prache *et al.* (2002) for bovine meat.

Potential tracers: how do they work?

Two types of tracers may be used, those that come directly from the food (direct tracers such as vegetal biomarkers) and those that derive from the animal metabolism (indirect tracers). For example as the alimentation affects the fatty acid composition of meat and milk, this composition can be useful in providing pertinent information concerning the food given to herbivores.

Vegetal biomarkers

Vegetal biomarkers are compounds that are not synthesized by animals and whose occurrence in the animal products or tissues is unambiguously due to the food they have eaten. The plant pigments carotenoids and the secondary metabolites terpenes are examples of such compounds among other candidates like flavonoids. These micronutrients are stored in the animal's fat after absorption and are thus found in milk and meat.

Lutein is the only carotenoid stored in the adipose tissue of sheep and goats (Yang *et al*., 1992; Prache *et al*., 2003b). This is of interest, because this pigment is present in high levels in green herbage (9-14 mg/100g fresh weight; Prache *et al*., 2003b), and it could therefore be an excellent biomarker of grass-feeding. In fact, some vegetables (spinach and parsley) contain around 9-12 mg/100 g fresh weight, but they do not really contribute to sheep or goat diet. The carotenoid found in maize, i.e. zeaxanthin, is not stored by ruminants. All grass conservation methods alter the forage carotenoid content. Compared to the initial level in fresh grass, the concentration decreases by about 60% for dehydrated forage and wilted silage, 70% for barn-dried hay, 80% to 90% for field-cured hay. The carotenoid content of the forage also sharply decreases with conservation duration.

Terpenes represent a large chemical class of molecules almost exclusively synthesized by plants (monoterpenes, sesquiterpenes and their oxygenated derivatives). Terpenes in grassland plants greatly vary according to the botanical family: most Apiaceae, certain Asteraceae and Lamiaceae contain great amounts and wide diversities of terpenes, whereas Poaceae contain only the most common terpenes (Cornu *et al*., 2001b). As natural grassland flora is a component of the "terroir", its terpene fingerprint could be valuable to decode. Actually, terpenes have been successfully used to recognise the animal diet, and also to localise the geographical origin of pasture-fed animals (Fernandez *et al*., 2003). Terpenes of the forage eaten by dairy cows are found in milk (Dumont and Adda, 1978) and meat (Larick *et al*., 1987), and their transfer from forage into milk is immediate (Viallon *et al*., 2000). Milks obtained on pasture contain much higher amounts and diversities of terpenes than milks obtained with conserved forages (Cornu *et al*., 2002).

Metabolic tracers

Suzuki and Bailey (1985) and Young *et al*. (1997) reported that 2,3-octanedione was an excellent marker of grass-feeding. This compound is related to the abundance in leafy plants of both the enzyme lipoxygenase (absent in corn) and its substrates linoleic and linolenic acids, which are mixed together during mastication.

Some authors reported that 3-methylindole (skatole) was present at higher concentrations in the fat from lambs grazing grass compared to those fed concentrates (Young *et al*., 1997). Skatole arises from tryptophan degradation. According to Sheath *et al*. (2001), the high protein/non-fibrous carbohydrate ratio typical of grass diets enhances protein deamination by rumen microbes and would therefore be responsible for high levels of skatole in fat from ruminants fed grass. Other studies however, did not confirm this result (Priolo *et al*., in press). These conflicting results may reflect differences in the breed and precocity of animals used and in the site of measurement (perirenal vs subcutaneous fat).

The fatty acid composition of meat and milk may also be useful to give pertinent information concerning the food given to animals. Grass lipids contain a high proportion of the unsaturated linolenic acid (C18: 3n-3), a compound that is not synthesized by animals. On the other hand, grain-based concentrate diets contain a high proportion of linoleic acid (C18: 2n-6). Part of the dietary linolenic acid escapes ruminal hydrogenation. Animals raised on pasture therefore have a higher

proportion of this acid in their lipids compared to stall-fed animals (Wood and Enser, 1997). So, the (n-6)/(n-3) ratio in phospolipids may be of interest to discriminate grass-fed from grain-fed lambs. Two derivates of linolenic acid, the eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) are restricted to the phospolipid fraction and are also generally at higher concentrations in grass-fed animals compared to stall-fed (Priolo *et al*. 2001). Aurousseau *et al*. (in press) raised Ile de France lambs at pasture or in stall and found that the (n-6)/(n-3) ratio in phospolipids made it possible to perfectly distinguish lambs fed on pasture from those given concentrates. A similar result was found by Priolo *et al*. (unpublished data) who perfectly discriminated lambs fed fresh sulla (*Hedysarum coronarium* L.) from those fed concentrates by using the (n-6)/(n-3) ratio in total lipids. This ratio was increased threefold in lambs given concentrates compared to those fed grass. However, linseed is also particularly rich in linolenic acid, and its inclusion in grain-based concentrate may affect the reliability of the discrimination based on the meat fatty acid composition.

Global approaches

It has recently been demonstrated that the spectral characterization of meat (m. *longissimus dorsi*) by using visible and near infrared reflectance spectroscopy (NIRS) was of interest to differentiate between beef meat samples that originated from two different feeding systems (diet based on 100% pastures vs diet based on maize silage) (Cozzolino *et al.,* 2002). Samples of meat were scanned in a NIRS monochromater instrument in a reflectance mode. Muscle authentification was performed by Principal Component Analysis and Soft Independent Modelling of Class Analogy. Qualitative analysis of optical information through this analysis showed differences in muscles resulting from the two different feeding systems.

Breakthroughs in the development of the functional genomics approach may also be promising. Given that nutrients regulate metabolic activity by modifying gene expression in herbivores, it is likely that the gene expression will be useful to develop new methods of traceability of grass-feeding (Eggen and Hocquette, 2004). However, this approach is just emerging and its experimental evaluation is still virtually non existent.

Discrimination of standard contrasting diets

The first step of our experimental evaluation of potential tracers was to investigate the discrimination reliability of these tracers in contrasting feeding diets conditions. In ovine meat, we compared lambs either fed exclusively grass at pasture (besides maternal milk) or stall-fed a diet containing 85% concentrate and 15% hay. The feeding level of stall-fed lambs was adjusted to achieve similar growth patterns in both treatments until slaughter. In these conditions, carotenoids in the blood and in the perirenal fat, terpenes and 2,3-octanedione in the fat and fatty acid composition of the meat all made it possible to distinguish between grass-fed and stall-fed animals. Grass-fed lambs accumulated 5 to 6 times more carotenoids in their blood, 2.4 to 4.1 times more lutein in their perirenal fat (Prache and Theriez, 1999, Priolo *et al*., 2002, Prache *et al*., 2003a and b), and 25 times more 2,3-octanedione in their fat than stall-fed lambs (Priolo *et al*., in press).

In this experiment, lambs fed the stall diet ingested about 3% of the carotenoid intake of the grazing lambs (Prache *et al*., 2003a). Carotenoid in blood was used successfully to discriminate grazing from stall-fed lambs, although large between-animal variability was observed (Prache and Theriez, 1999, Prache *et al*., 2003a). Moreover, Prache and Theriez (1999) pioneered an original method to discriminate carcasses of grazing *vs.* concentrate-fed lambs from the analysis of the reflectance spectrum of the fat in the zone of light absorption by carotenoids. Figure 2 in Prache *et al.* (2003b) gives an illustration of this analysis and of the calculation of the index of traceability we use to quantify light absorption by carotenoid. This method was used successfully in ovine meat (Prache and Theriez, 1999, Priolo *et al*., 2002, Prache *et al*., 2003b). It was also used quite successfully for ovine milk. In this latter case, two treatments were compared: a diet containing maize silage, concentrate and oats hay *vs.* 4h-grazing per day + concentrate + hay indoors. The index of traceability was different between treatments; there was some overlapping for 16% of samples, but it is likely that the reliability of the discrimination would be further improved with higher grazing times. This method, for which there is a patent applied, has the advantage of being noninvasive, fast and easy to use in the meat industry. It has been further generalized to bovine meat, milk and cheese (Prache *et al*., 2002).

Persistence of the tracers and implications for traceability purposes

Grazing lambs may be concentrate-finished because of a grass shortage. The effect of concentrate finishing after grazing on the different tracers' concentration in sheep tissues and its significance for traceability purposes have been recently investigated (Prache *et al.*, 2003a and b for carotenoid pigments; Priolo *et al.*, 2003 for fat volatiles). These authors compared the following feeding treatments: grazing, feeding a concentrate-based diet, concentrate-finishing period after grazing. The stall-finishing period lasted on average 36 days (minimum and maximum 15 and 63 days), and during the finishing period, lambs deposited on average 6.6 kg liveweight (minimum and maximum 1.6 and 12.1 kg liveweight). Carotenoid in blood and fat and 2,3-octanedione in fat decreased curvilinearly with the interval from starting on the stall diet after pasture, according to a decreasing exponential model $Y=a \times e^{(-b \cdot day)}$ (eq.1) A semineperian logarithmic representation of this model is useful [$Log\ Y = a - (b \cdot day)$] (eq. 2), b being the deceleration parameter.

Among the fat volatiles tracers, 2,3-octanedione proved to be the most interesting to provide reliable information concerning the duration of the concentrate-finishing period. Actually, the terpene profile of the stall-finished grazing lambs did not differ from the terpene profile of the exclusively stall-fed animals, suggesting that the persistence of these compounds was low. It is worth noting that the residual standard deviation of the model of persistence of 2,3-octanedione (eq. 2) is low, suggesting a low interference of between-animal variability.

The persistence of carotenoid in blood was low: After 4 to 13 days on the stall diet, depending on the initial level of plasma carotenoid content (between-animal variability), plasma carotenoid content of concentrate-finished grazing lambs fell to the values of stall-fed lambs (Figures 4 and 5 in Prache *et al.*, 2003a).

The effect of stall-feeding duration on the carotenoid content of the fat in concentrate-finished grazing lambs is mediated via a dilution of existing fat with whiter fat rather than through pigments coming out of the fat. The model we performed predicts that the mean absolute value of the index of traceability in stall-finished grazing lambs decreases to a level similar to that of the stall-fed lambs after 10.7 kg LW gain on average on the stall-feeding diet. This corresponds to a duration of the stall-finishing period of about 50 days when lamb ADG is 216 g/day (our study). However, because of the between-animal variability in carotenoids absorption, the only use of the reflectance spectrum of the fat may cause some concentrate finished grazing lambs to be classified as stall-fed lambs shortly after the beginning of the stall period, whereas others may be considered as grazing lambs for a long period of time. However, the combined use of plasma carotenoid content at slaughter together with the reflectance spectrum of the fat improves reliability in the discrimination of grass-fed, concentrate-fed and concentrate-finished grazing lambs, by taking advantage of the differences in the rate of reduction in carotenoid concentration in blood and fat.

More generally, our data suggest that the combined use of different tracers and different tissues may be valuable to trace the stall-feeding duration. Actually, the deceleration parameter varies according to the tracer and the tissue: it is 0.2124 and 0.0259 for carotenoid in blood and fat and 0.0369 for 2,3 octanedione in fat. Taking advantage of the differences in these persistence profiles by the combined use of different tracers and tissues may thus improve our ability to predict and control the finishing period duration. This may be of interest to guarantee objectively that the specification commitments concerning the stall-finishing of grazing animals have been correctly fulfilled.

Conclusions

Research on traceability of grass-feeding in herbivores' meat and milk takes place within a general context of increased consumer demand for information and guarantees concerning the mode of production of animals, and particularly the animal feeding regimes. Within this context, there is a need for controlling tools, in order to guarantee objectively that the specification commitments have been correctly fulfilled. The first results indicate that it is possible to trace grass-feeding in herbivores' meat and milk by using analytical methods that quantify direct or indirect tracers in the product or the animal tissues. Results also show that the combined use of the different tracers is of interest. The cost and potentiality to be readily implemented are variable among methods. The spectrocolorimetric method aiming at quantifying carotenoid pigments concentration in the fat can be used rapidly in the

industry. Other methods, that are expensive, can only be used at present on a small number of samples, but their potential use may dissuade from eventual fraud. These methods were developed using a relatively small number of animals. They are currently being validated with a much larger number of animals, a validation procedure being essential considering the sometimes high between-animal variability. Some approaches are just emerging and need further experimental evaluation.

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